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Polybrominated Dibenzo-*p*-Dioxins and Dibenzofurans: Literature Review and Health Assessment

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Polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs) occur as trace (ppb) contaminants in brominated flame retardants and are produced during combustion of these chemicals. They are also formed when organics are incinerated in the presence of bromine, e. g., in municipal and industrial incinerators and in internal-combustion engines. Combustion of organics in the presence of both bromine and chlorine results in the formation of mixed (i.e., bromo, bromo/chloro and chloro) halogenated dibenzo-*p*-dioxins and dibenzofurans (HDDs and HDFs). There are 4600 potential mixed congeners. The biological effects of PBDDs and PBDFs are similar, if not identical, to those of PCDDs and PCDFs. Both groups of compounds induce hepatic aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*o*-deethylase (EROD) in rats and cause wasting and thymic atrophy in rats and guinea pigs. Tetrabrominated dinenzo-*p*-dioxin (TBDD) and dibenzofuran (TBDF) are reproductive toxins in mice and produce skin lesions in the rabbit-ear acnegenic test. The brominated compounds appear to bind to the same cytosolic receptors believed to mediate the toxicities of the chlorinated analogs. When compared on a molar-concentration basis, the brominated compounds are equipotent to the chlorinated analogs. TBDD is absorbed after oral, dermal, or intratracheal administration in rats, stored in the liver and adipose tissue, and eliminated in the feces through biliary excretion. The biological half-lives of the brominated compounds appear to be somewhat shorter than those of the corresponding chlorinated species. The brominated compounds, like their chlorinated congeners, have the potential to cause dermal, hepatic, and gastrointestinal toxicities in humans. Further, because the carcinogenic potential of the chlorinated compounds in humans has been established, the brominated compounds should be considered human carcinogens.

Introduction

Relatively little is known about the environmental and toxicologic significance of polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDDs and PBDFs). These chemicals have only recently been identified as potential environmental pollutants. The chlorinated analogs of these chemicals have been studied for years.

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs) are recognized as relatively low-level but toxicologically significant environmental contaminants. These materials are not manufactured commercially, but some appear as impurities in certain products such as herbicides and fungicides. In addition, significant amounts of these important chemicals are produced in thermal reactions, such as those in municipal and industrial waste incineration, accidental fires, and the burning of automotive fuels that contain chlorinated additives. In fact, the generation of PCDDs and PCDFs is considered likely whenever organic materials and a suitable chlorine source are combusted under oxygen deficient conditions.

The experimental and clinical toxicology of the PCDDs and PCDFs have been extensively studied and well documented; therefore, they will not be extensively reviewed in this paper. The most potent PCDDs and PCDFs are the 2,3,7,8-chloro congeners, but all are considered to possess significant toxicologic potential (*1–3*). Human exposures to PCDDs and PCDFs have been associated with dermal, hepatic, gastrointestinal, and possibly neurologic toxicity. Also, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been shown to be a potent carcinogen in laboratory rodents, and Fingerhut et al. (*4*) have reported an association between high cumulative doses and excess cancer mortality among occupationally exposed humans. PCDDs and PCDFs have also been shown to produce immunologic, reproductive, and developmental toxicities in laboratory animals.

There are only limited quantitative data regarding the environmental occurrence of PBDDs and PBDFs, and it is impossible at this time to project the magnitude of health hazard, if any, that may attend the accidental and/or incidental production of these compounds. However, their structural similarities to the PCDDs and PCDFs make them logical subjects for environmental and toxicologic investigation. The purpose of this communication is to present a preliminary review of the literature concerning the human health hazard potential of PBDDs and PBDFs.

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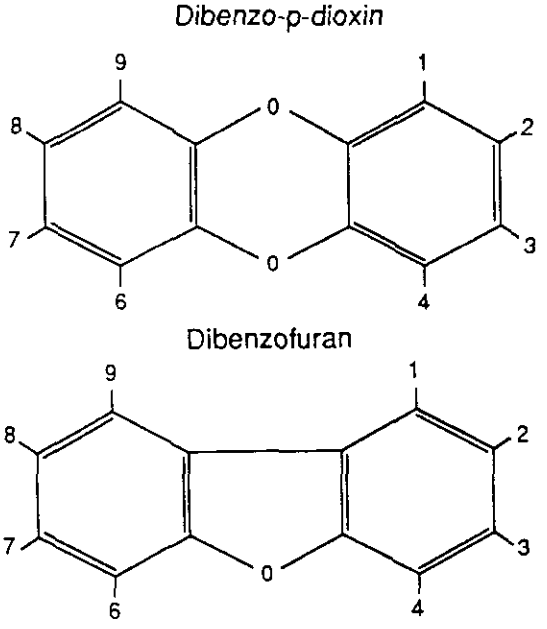

FIGURE 1. Structural formulas of unsubstituted dibenzo-*p*-dioxin and dibenzofuran.

Formation, Occurrence, and Stability of PBDDs and PBDFs

It has been recognized that the environmental presence of halogens such as bromine, and to a lesser extent iodine and fluorine, has the potential of giving rise to the formation of additional polyhalogenated dibenzo-*p*-dioxins and dibenzofurans (PHDDs and PHDFs) under the same conditions as PCDD and PCDF production (*5*). The structural formulas of unsubstituted dibenzo-*p*-dioxin and dibenzofuran and the numbering of the carbon atoms are shown in Figure 1. The halogenated compounds discussed in this review contain bromine (or chlorine) at the positions indicated in their names.

PBDDs and PBDFs can be produced by the pyrolysis of a variety of brominated flame retardant chemicals (*6–17*) and/or flame-retarded polymeric materials. Under conditions in which a source of chlorine is also available, bromo/chloro dibenzo-*p*-dioxins and furans are most likely produced. Because chlorinated derivatives are preferably formed during pyrolysis, fully brominated compounds will only rarely be produced, and the mixed compounds will predominate (*5*). In this regard, while there are only 210 possible congeners containing a single halogen, a total of 4600 discrete bromo, chloro, and mixed halogen derivatives are possible.

Halogenated dibenzo-*p*-dioxins and dibenzofurans are also produced during the incineration of organic materials in the presence of halogens. The specific halogenated congeners and amounts formed depend on the reactivity of the precursors, product stability, and the Br/Cl ratios. While assuming other factors to be equal, Buser (*5*) computed the probable distribution of HDDs and HDFs formed during organic incineration in the presence of various Br/Cl ratios. When Br/Cl = 1, the mixed halogenated derivatives are expected to predominate, with only small amounts of products containing a single halogen (i.e., either bromine or chlorine) being formed. In the presence of a 10-fold excess of chlorine relative to bromine, the fully chlorinated compounds are likely to predominate, but mixed derivatives will still be present in small amounts.

These predictions are of practical import because Br/Cl ratios almost certainly vary from site to site. For example, in municipal incinerators, the sources of chlorine normally far exceed sources of bromine. Under these conditions, polychlorinated derivatives would be expected to predominate. The widespread use of brominated flame retardants in commercial products such as carpets, textiles, and plastics and their inevitable disposal, at least in part by incineration, afford a potentially significant source of bromine for the generation of bromo and bromo/chloro dibenzo-*p*-dioxins and dibenzofurans.

The results of analyses of environmental samples are consistent with Buser's prediction of the production of mixed derivatives. The analysis of ash from municipal incinerators (*18–21*) as well as automobile exhausts (*5,7,22*) has revealed the presence of primarily bromo/chloro dibenzo-*p*-dioxins and dibenzofurans.

Neupert et al. (*23*) examined the stability of PBDDs and PBDFs under laboratory conditions. Solutions of 2,3,7,8-TBDD or TBDF in toluene were exposed to fluorescent light, at laboratory temperature, 24 hr/day for up to 6 days. Both compounds underwent degradation, with the furan appearing to degrade somewhat faster than the dioxin. After 15 days, the concentrations of the solutions had declined to less than 35% of original. The major decomposition products were debrominated compounds containing from one to three bromine atoms. Samples of penta-, hexa-, and octa-BDDs and BDFs were similarly studied, and the rate of degradation was directly proportional to the degree of bromination.

Buser (*24*) compared the photolytic decomposition of bromo and bromo/chloro dibenzo-*p*-dioxins and dibenzofurans to that of the corresponding polychloro compounds. When dissolved in toluene, both the polybromo and bromo/chloro compounds underwent decomposition more rapidly than the corresponding polychloro derivatives. These results suggest that the brominated compounds may be less persistent than the chloro derivatives.

It should be noted, however, that environmental PBDD/Fs may be less susceptible to photodegradation than are laboratory samples. Studying the degradation of PCDDs and PCDFs adsorbed to fly ash, Koester and Hites (*25*) found no significant photodegradation, although the compounds are susceptible to photodegradation when dissolved in various organic solvents. These results may suggest that photodegradation may not be a significant mechanism for the removal of the chlorinated compounds from the environment and may also be less effective than anticipated in removing the bromo and bromo/chloro derivatives. Similar experiments with the brominated compounds have yet to be conducted.

Toxicity in Experimental Animals

Single-dose Studies

Moore et al. (*26*) reported on the comparative toxicities of 2,3,7,8-TCDF and TBDF administered by gastric intubation to male Hartley guinea pigs. TBDF was dissolved in corn oil and administered in single doses of 0, 0.47, 1.58, 4.74, or 15.81, μg/kg.

These doses of the bromo congener are the molar equivalents of 0, 0.3, 1.0, 3.0, or 10 μg/kg of the chlorinated congener TCDF. Actual doses of TCDF administered in this study were 0, 1.0, 5.0, 10, or 15 μg/kg. Animals were observed until death or for 30 days after dosing. Gross signs of toxicity produced by the two HDFs were similar and included decreased body weight gains after the lower doses (1.0 μg/kg for TCDF and 4.74 μg/kg for TBDF) and prompt body weight loss after the higher doses (10 μg/kg for TCDF and 15.8 μg/kg for TBDF). The minimum lethal dose of TBDF may have been somewhat lower than that of TCDF (4.74 μg/kg for TBDF versus 10 μg/kg for TCDF), but the number of doses studied and animal group sizes were too small to allow meaningful comparisons.

TCDD is known to produce a spectrum of toxicological effects in laboratory animals, with marked quantitative differences between species. Poland and Knutson (*1*) reviewed the toxicity of TCDD and related aromatic hydrocarbons and noted that the toxic syndrome produced in laboratory animals by these compounds frequently includes wasting, lymphoid involution, hepatotoxicity, epidermal changes, gastric lesions, and urinary tract hyperplasia. Mason et al. (*27*) demonstrated that a series of polybrominated and bromo/chloro dibenzo-*p*-dioxins produced body weight loss and thymic atrophy in immature male Wistar rats. The compounds studied (1,3,7,8-TBDD; 2,3,7,8-TBDD; 1,2,3,7,8-PeBDD; 1,2,4,7,8-PeBDD; 2,3-dibromo-7,8-di-CDD; and 2-bromo-3,7,8-tri-CDD) were administered IP (dose ranges studied were not specified), and animals were observed for 14 days. Each of the compounds reduced body weight and caused thymic atrophy. The ED_{50} for body weight loss ranged from 1.24×10^{-8}M for 2,3-dibromo-7,8-di-CDD to 2.52×10^{-4}M for 1,3,7,8-TBDD. The order and magnitude of potency for the induction of thymic atrophy were similar.

Like the more intensively studied CDDs and CDFs, the brominated derivatives are also potent inducers of microsomal monooxygenase activity. The bromo and bromo/chloro dibenzo-*p*-dioxins are capable of inducing hepatic, aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*o*-deethylase (EROD) either *in vitro* (rat hepatoma H-4-II E cells) or *in vivo* (rats). Mason et al. (*27*) compared the relative abilities of TCDD and TBDD to bind to rat liver cytosolic receptors (hydroxylapatite receptor binding assay) and to induce AHH and EROD *in vitro*. The affinities of the compounds for receptor binding sites appeared to be equivalent, and the chlorine derivative appeared to be somewhat more potent as an inducer of AHH and EROD. The results of *in vivo* experiments demonstrated enzyme induction by a series of bromo and mixed dibenzo-*p*-dioxins. Nagao et al. (*28*) and Schulz-Schalge et al. (*29*) have reported similar *in vivo* hepatic enzyme-inducing potencies of chlorinated and brominated compounds.

There appears to be a high degree of correlation between the ability of PHDDs and PHDFs to induce AHH activity *in vitro* and to produce *in vivo* toxicity. Safe et al. (*30*) studied a series of 27 PCDD, PCDF, PBDD, and PCB congeners and reported high correlation coefficients ($r > 0.9$) between *in vitro* enzyme induction and the ED_{50} for either body weight loss or thymic atrophy in rats. The specific congeners studied and doses tested were not specified in the publication.

Repeated-Dose Studies

Hardy et al. (*31*) administered TBDF to male and female Sprague-Dawley rats 5 days/week for 4 weeks. TBDF was administered as a corn oil gavage at 0, 1, 10, 50, 150, or 500 μg/kg/day (0.002 to 1.03 μmole/kg/day). All animals that received 50 μg/kg/day or less survived for the scheduled duration of the study. All animals administered 500 μg/kg/day died or were sacrificed *in extremis*; 70% of the animals receiving 150 μg/kg/day died. Animals dosed with 50 μg/kg/day or more exhibited decreased body weight gains. Mean thymus weight (relative to body weight) was decreased in males by 150 μg/kg/day and in females by 10 and 50 μg/kg/day. Male kidney and testes-to-body-weight ratios were significantly increased by the 50 and 150 μg/kg/day doses. Liver, kidney, spleen, adrenal gland, heart, and thymus from animals in the control, and 1, 10, and 50 μg/kg/day groups were examined microscopically. Hepatic lesions were characterized as panlobular hypertrophy of the hepatocytes with associated panlobular hepatocyte vacuolation and focal necrosis.

Löser and Ivens (*32*) conducted a 13-week study of the toxicity, storage, and elimination of TBDD in male and female Wistar rats. Animals were administered TBDD by daily gavage in arachis oil (0.01, 0.1, 1.0, 3.0 or 10 μg/kg; 0.00002 to 0.02 μmole/kg) for 91 days. The lowest lethal dose was 3.0 μg/kg for both sexes, and all rats in the 10 μg/kg group died or were killed *in extremis* before the scheduled termination of the study. The authors stated that animals dosed with either 0.01 or 0.1 μg/kg exhibited no treatment-related signs of toxicity; however, 0.1 μg/kg (or higher) produced decreases in serum thyroxin in both sexes. There was also an increase in triiodothyronine in animals dosed with 1 or 3 μg/kg. The major effects noted in the 3 and 10 μg/kg groups included decreased body weight gain, poor general health, icterus, and, after 12 weeks, increased plasma alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase in the 3 μg/kg group animals that survived to the scheduled termination of the study.

Toxic Effects in Humans

Reports describing toxic effects in humans attributable to PBDDs or PBDFs were not found. However, given the similarities between the PCDDs, PCDFs, PBDDs, and PBDFs, it is reasonable to predict that the brominated compounds will produce the same spectrum of effects in humans as produced by the chlorinated compounds.

Despite a wide variety of toxicological manifestations exhibited by laboratory animals after exposure to the PCDDs and PCDFs, toxicological effects in humans (other than the production of chloracne) are not well documented (*1–3*). Several reports suggest possible immunosuppression in humans, but these effects have not been unequivocally proven [reviewed by Lorenzen and Okey (*33*)].

A common element found in nearly all pathological lesions associated with PCDDs and PCDFs is the presence of the genetically segregated Ah receptor, and the current consensus is that toxicity of either class of chemicals is mediated through this receptor. Lorenzen and Okey (*33*) have demonstrated the presence of this receptor in cells from the human tonsil. The receptor was found to bind TCDD and to be transformed to a nuclear binding form. Further studies on the interactions of polyhalogenated compounds with the human Ah receptor might provide useful insights into the human toxicological potential of the brominated compounds.

Developmental Toxicity in Experimental Animals

PBDDs and PBDFs are potent developmental toxins in mice. The evidence indicates that compared to chlorinated analogs, replacement of chlorine with bromine may reduce potency somewhat, but does not appear to alter the mechanism through which this class of chemicals exerts its developmental effects (*34*).

TCDD is a potent developmental toxin in mice, causing cleft palate and hydronephrosis at dose levels that produce no overt maternal or fetal toxicity (*35*). Birnbaum et al. (*34*) studied the effect of substituting bromine for chlorine in the TCDD molecule on teratogenic potency. In addition to studying the brominated analog of TCDD, these workers also assessed the developmental effects of TBDF, 1,2,3,7,8-penta-BDF (1PeBDF), and 2,3,4,7,8-penta-BDF (4PeBDF). Corn-oil gavage doses administered to pregnant C_{57}BL/6N mice on gestation day 10 ranged from 0 to 192 μg/kg (up to 0.38 μmole/kg) for TBDD and from 0 to 4,000 μg/kg for TBDF, 1PeBDF, and 4PeBDF. All doses of each compound significantly increased liver weights in the dams but produced no other evidence of maternal toxicity. TBDD and TBDF caused dose-related increases in fetal weights, and 500 μg/kg or more of TBDF significantly increased embryo/fetal mortality.

The induction of hydronephrosis was the most sensitive measure of developmental toxicity. The incidence was increased by each compound, and ED_{50} doses were estimated to be TBDD, 9 μg/kg; TBDF, 12 μg/kg; 1PeBDF, 340 μg/kg; and 4PeBDF, 4347 μg/kg. The authors estimated that the ED_{50} dose for TCDD to produce hydronephrosis was 4.0 μg/kg. Thus, TBDD, the most potent inducer of hydronephrosis of the brominated congeners tested, was estimated to be roughly one-half as potent as TCDD in this regard. When molar doses are compared, TBDD and TCDD were approximately equipotent. All of the PBDDs and PBDFs also caused significant increases in the incidence of cleft palate. Estimated ED_{50} values were: TCDD, 15.3 μg/kg; TBDD, 65.1 μg/kg; TBDF, 153.5 μg/kg; 1PeBDF, 4,087.6 μg/kg; and 4PeBDF, 3024.3 μg/kg. Probit plots of the cleft palate incidences revealed a common slope, which the authors interpreted to suggest that the compounds may produce their effects through the same mechanism.

Nagao et al. (*36*) compared the cleff-palate-inducing effects of TBDD in NMRI mice. Animals were dosed with from 5 to 90 μg/kg of either TBDD or TCDD on day 9 of pregnancy. Both compounds produced only minimal evidence of maternal toxicity (increased liver-to-body-weight ratios at all dose levels). Neither TBDD nor TCDD affected fetal weights or numbers of viable fetuses per litter: There were, however, clear dose-related increases in the incidences of cleft palate in pups whose dams had been dosed with either compound. The ED_{50} value for TBDD was estimated to be 61.7 μg/kg as compared to 65.1 μg/kg reported by Birnbaum (*34*). The ED_{50} of TCDD to induce cleft palate was computed to be 24 μg/kg. No studies of other reproductive effects of PBDDs or PBDFs in laboratory animals or humans were reported.

Mutagenicity and Other Indications of Genotoxicity

The International Agency for Research on Cancer (*37*) reviewed the genotoxic potential of TCDD and reported that the compound did not produce dominant lethal mutations, chromosomal aberrations, micronuclei, or sister chromatid exchanges in rodents treated *in vivo*. It was mutagenic to mouse lymphoma cells but did not induce unscheduled DNA synthesis in rat hepatocytes *in vitro*. TCDD was not mutagenic to bacteria.

No reports relating directly to the genotoxicity of PBDDs or PBDFs were found. Since the *in vivo* and *in vitro* biological actions of TCDD and TBDD appear to be similar, information on the genotoxicity of TCDD does not suggest an imminent genotoxic threat from the brominated compound. Studies on the bromo and bromo/chloro compounds are clearly indicated.

Carcinogenicity

Experimental or epidemiological studies of the carcinogenic potential of the PBDDs and PBDFs have not been reported, and we are unaware of studies in progress. 2,3,7,8-TCDD is clearly carcinogenic in laboratory animals (*37*), and there is a growing body of evidence indicating that it is also carcinogenic in humans (*4*). For these reasons it is prudent to assume that 2,3,7,8-TBDD is probably carcinogenic also.

Absorption, Excretion, Distribution and Metabolism

In experiments in which TBDD has been compared to TCDD, the absorption, elimination, and distribution of the two compounds appear to be similar. Although not studied simultaneously, the elimination of TBDD and TCDD appears to be similar also.

Absorption and Elimination

The studies that have been reported on TBDD to date show that, in rats, the compound is absorbed after oral, dermal, or intratracheal administration, and the primary route of elimination is the feces, with biliary excretion appearing to be the major source of compound.

Diliberto et al. (*38*) studied the absorption and excretion of orally administered [^{3}H]TBDD in male F344 rats. Doses studied included 0.001, 0.01, 0.1. or 0.5 μmole/kg. These doses are equivalent to 0.5 to 250 μg/kg. The major route of excretion was the feces. Seventy-two hr after administration, fecal excretion accounted for 30–80% of the administered dose. Urinary excretion was not described. At the termination of the experiment, the majority of the body burden was located in liver and fat. Concentrations in these tissues for animals exposed to the two lower doses were directly related to size of the dose. Higher doses, however, were associated with a decreased percentage of the administered dose remaining in tissues. The dose-related increase in fecal excretion and decrease in relative tissue residues at higher doses suggest that, at the doses studied, absorption of TBDD from the gastrointestinal tract is nonlinear.

Diliberto et al. (*39*) reported absorption and excretion data after the administration of [^{3}H]TBDD to male F344 rats by intratracheal instillation. Three days after the instillation (1.0 nmole/kg in 250 μL ethanol: Emulphor: water), 22% of the administered radioactivity was found in adipose tissue and 18% was

in the liver. The major route of excretion was the feces, which accounted for 37% of the administered radioactivity within 72 hr.

Jackson et al. (*40*) reported that approximately 12% of a dermally applied dose of [^{3}H]TBDD (1.0 nmole/kg dissolved in 60 μL of acetone) was absorbed during 72 hr. Approximately 13% of the radioactivity remained in the application site, and major tissue depots were found to be liver and adipose tissue. The liver to adipose tissue concentration ratio was 3:2. Elimination (only 17% of the absorbed dose) was primarily via the feces. The authors stated that at equimolar doses, only 30–40% as much TBDD was absorbed through rat skin as was TCDD (*40*).

Kedderis et al. (*41,42*) estimated that the whole/body half-life of TBDD was 2–3 weeks in F344 rats after the IV injection of 0.001 or 0.1 μmole/kg (0.5 or 50 μg/kg). By 56 days after administration, 50% of the low dose and 70% of the high dose had been excreted in the feces. Urinary excretion accounted for 4.5% of the administered low dose and 7.6% of the administered high dose during the same period. Kedderis et al. (*42*) also compared the biliary excretion of IV-administered [^{3}H]TBDD and [^{3}H]TCDD by male F344 rats. During the initial 8 hr after the administration of either 0.001 or 0.1 μmole/kg of TBDD, 6–8% of the administered radioactivity was excreted in the bile. Similar results were obtained when equimolar doses of TCDD were administered.

Distribution

Absorbed TBDD is quickly distributed from the blood to skeletal muscle, skin, liver, and adipose tissue in rats. Highest tissue concentrations are achieved in the liver, with a gradual redistribution to adipose tissue. The compound is most persistent in adipose tissue and skin, with half-lives of approximately 58 days in both tissues.

Single-Dose Studies. Kedderis et al. (*43*) studied the disposition of [^{3}H]TBDD in male F344 rats for 56 days following the intravenous injection of either 0.001 or 0.1 μmole/kg (0.5 or 50 μg/kg). After the low dose, TBDD was rapidly cleared from the blood, with levels declining to less than 2% of the administered dose within 1 day. Hepatic radioactivity peaked at 7 hr then declined (half-life, 17 days). Adipose tissue concentrations rose through day 14, then slowly declined (half-life, 58 days). Approximately 10% of the administered radioactivity was found in the skin at 7 hr and a biphasic elimination from this tissue resulted in a long half-life similar to that of adipose tissue (58 days). Concentrations in muscle generally paralleled those in the blood, peaking early and declining rapidly.

Although the high dose (0.1 μmole/kg) was 100 times greater than the low-dose, liver concentrations were almost 700 times greater in the high- than in the low-dose groups at 56 days. These results indicate dose-related differences in deposition. In addition, the relationship between radioactivity in liver and adipose tissue exhibited both time- and dose-dependent behavior. At the low dose, liver-to-adipose-tissue concentration ratios declined from a high of 30 on day 1 to approximately 1 on day 14. By day 56, adipose tissue contained about 6-fold more radioactivity than liver. These results suggest a continued redistribution of TBDD from liver to adipose tissue. By way of contrast, 56 days after 0.1 μmole/kg, concentrations of radioactivity in the liver were still two to three times greater than those in adipose tissue.

Repeated-Dose Studies. Ivens et al. (*44*) administered daily doses of 0.01, 0.1, 1.0, 3.0, or 10.0 μg/kg (0.00002, 0.0002, 0.002, 0.006 or 0.02 μmole/kg) of TBDD as a daily arachis oil gavage to male Wistar rats for 91 days. Concentrations of TBDD in liver and adipose tissue were determined at several intervals during the dosing period and at 100, 121, and 184 days after cessation of administration (recovery period). Tissue concentrations of TBDD increased in dose- and time-related fashions throughout the period of administration. Liver levels of TBDD declined promptly during the recovery phase. Within 93 days, hepatic levels were reduced by 98% of the levels produced by 91 consecutive days of dosing with 1 μg/kg. Adipose tissue levels declined more slowly, with no significant decrease during the initial week of recovery and an 86% decline after 93 days of recovery. These results are suggestive of multiphasic elimination of TBDD from the liver and adipose tissue. The authors noted that tissue concentrations achieved after the daily administration of 1.0 μg/kg of TBDD for 91 days were approximately half those reported by Kociba et al. (*45*) after the administration of the same dose of TCDD 5 days a week for 13 weeks. Ivens et al. (*44*) suggested that this observation may indicate that the half-life of TBDD is shorter than that of TCDD.

Metabolism

Although little information on the biotransformation of the PBDDs and PBDFs was found, it is clear that TBDD undergoes metabolism in F344 rats. Kedderis et al. (*42, 43*) reported that 80–90% of biliary and fecal radioactivity excreted by rats administered [^{3}H]TBDD IV was in the form of TBDD metabolites. No parent compound was found in the bile, but 10–20% of the radioactivity found in the feces on days 1 to 3 was TBDD. The nature of the excreted radioactivity was examined by HPLC and found to elute before known TBDD, indicating increased polarity relative to the parent compound. The metabolites were not identified. HPLC analysis of ^{3}H extracted from liver showed that all radioactivity present eluted with the parent TBDD. Pretreatment with TBDD (0.1 μmole/kg by gavage 3 days before IV injection of 0.001 μmole/kg [^{3}H]TBDD) increased hepatic uptake of the radiolabel but had no effect on biliary excretion. Similar results were obtained when rats were dosed with equimolar doses of TCDD.

Bioaccumulation/Bioconcentration

Studies dealing directly with the bioaccumulation and bioconcentration of PBDDs and PBDFs are limited. However, the results of three reports suggest that the brominated compounds may exhibit less bioaccumulation and bioconcentration than their chlorine analogs. Neupert et al. (*23*) reported that a series of PBDDs and PBDFs were labile to artificial light. Under similar conditions the chlorinated TCDD and TCDF did not degrade. Ivens et al. (*44*) reported hepatic and adipose tissue accumulation of TBDD in rats during subchronic administration. Although both tissues accumulated the compound during administration, concentrations in both began to decline shortly after cessation of dosing. The rate of decline of the brominated compound from these tissues is faster than that of TCDD. These results suggest that the biological half-life of TBDD is likely to be less than that of TCDD. Kedderis et al. (*43*) estimated the terminal whole-body half-life of TBDD in rats to be 18 days after

a single high or low IV dose. This is similar to results reported by Rozman et al. (*46*) for TCDD but somewhat shorter than the 31-day half-life estimated for TCDD in the rat by Rose et al. (*47*).

Obviously, unequivocal statements regarding the bioaccumulation and concentration of the PBDDs and PBDFs cannot be made. It is likely that the number of bromine atoms on the molecules influences biological half-life. If this is the case, the bioaccumulation and concentration of the mixed halogen compounds will probably be different from that of either the completely brominated or chlorinated derivatives. Studies of bioaccumulation of PBDDs and PBDFs are required.

Ecological Effects

Effects on Organisms

Studies addressing the potential effects of PBDDs and PBDFs on environmental organisms were not found. Given the similarities between the chlorinated and brominated derivatives, however, it is reasonable to use data on the PCDDs and PCDFs to make an initial extrapolation to the PBDDs and PBDFs.

Mehrle et al. (*48*) tested TCDD and TCDF in rainbow trout (*Salmo gardneri*). Fish were exposed to the chemicals for 28 days, then transferred to uncontaminated water for a 28-day depuration period. The no-observable-effect concentration (NOEC) for TCDD was found to be less than 0.38×10^{-4} μg/L (the lowest concentration tested). Deaths occurred throughout the entire depuration phase, even with the lowest concentration. In a similar experiment with TCDF, the NOEC for survival was 17.9×10^{-4} μg/L and the NOEC for growth was 4.1×10^{-4} μg/L. Based on experiments conducted in mammalian systems (*34,44*), one would expect polybrominated congeners to be approximately half as toxic on a microgram per liter basis (essentially equipotent on a molar basis).

Tissue Residues

Kuehl et al. (*49*) analyzed chemical residues in Atlantic Ocean dolphins that had died during the 1987–1988 mass mortality. A total of 21 animals (14 bottlenose, 3 whitesided, and 4 common) were surveyed. In addition to a variety of pesticide residues, several PCDFs and 1,2,3,4,6,7,8-HpCDD were found. TCDD was not detected in the blubber of any animals, but the authors stated this might be a reflection of a relatively insensitive method of analysis (15 pg/g tissue). Tissue samples from three animals were also evaluated for the presence of unknown chemical contaminants. In addition to contaminants later identified as polybrominated biphenyls (PBBs, 17 ng/g lipid) and polybrominated diphenylethers (200 ng/g lipid), these authors reported the presence of several other polybrominated contaminants. One compound, with a molecular weight of 650 daltons, appears to contain both bromine and chlorine atoms. This molecular weight is not inconsistent with the presence of a polybrominated/chlorinated dibenzo-*p*-dioxin. Efforts to fully characterize the unidentified compounds are currently underway.

Mechanism of Toxicity

Based on a limited amount of testing, it appears that the PBDDs and PBDFs produce toxicty in laboratory animals that is qualitatively similar to that produced by the PCDDs and PCDFs. When *in vivo* exposures and *in vitro* concentrations of TCDD and TBDD are expressed in terms of micrograms, TCDD appears to be approximately twice as potent as TBDD. However, when doses and concentrations are expressed on the more appropriate molar basis, the two compounds appear to be equipotent.

Because of the similar chemical behavior of chlorinated and brominated derivatives, it seems possible that the two groups of chemicals may act through the same cytosolic receptor-mediated mechanism. Differences in potency and duration of action (if they exist) might be reflections of different rates of metabolism or different affinities for binding to the cytosolic receptor.

Receptor-Mediated Toxicity

There is compelling evidence that the toxic responses to TCDD and related HDDs and HDFs (including the brominated compounds) are mediated through interactions with cytosolic receptor(s), as summarized at the Banbury Conference on Dioxin (*50*). It has been proposed that the toxicologic actions of the HDDs and HDFs proceed through an initial binding of the toxicant to a cytosolic receptor protein. Given the chemical similarities between chlorine and bromine, it is reasonable to suspect that both bromine and chlorine congeners will interact with the same receptors. However, chlorine and bromine bond strengths as well as the relative sizes of the halogen atoms differ. These factors may influence receptor binding affinities as well as the relative susceptibilities of the molecules to enzymatic attack and biotransformation. Under these circumstances one would expect qualitatively similar effects to be produced by chlorinated and brominated compounds, with any quantitative differences being attributable to the nature of the halogen substituent.

Although not affording unequivocal proof, the results of some recent studies on the brominated derivatives are consistent with the suggestion that the brominated and chlorinated compounds interact with the same receptors. Both brominated and chlorinated derivatives induce hepatic AHH and EROD, with the chlorinated derivatives being more potent (*6,27,28,30,51*). Most recently, Birnbaum et al. (*34*) found that the teratogenic (cleft palate) dose–response curves for PBDDs and PBDFs in mice are parallel to those of TCDD. The authors noted that parallel dose–response curves may be suggestive of similar mechanisms of action.

Toxicity Equivalency Factor/TCDD Equivalent

The enormous number of polybrominated and mixed (brominated/chlorinated) dibenzo-*p*-dioxins and dibenzofurans makes the investigation of the toxicologic potential of even a small percentage of these compounds unrealistic. A similar dilemma exists in the case of the PCDDs and PCDFs. The toxicity equivalency factor (TEF) concept (*52,53*), which depends on a strong structure–activity relationship between the congeners and their ability to elicit a biological/toxic response in various *in vitro* and *in vivo* test systems allows the assignment of potency factors for individual members of a chemical group. The potency factor expresses the toxicity of a specific congener relative to that of a well-studied standard. In the case of PCDDs/PCDFs, the

standard chemical is TCDD. In estimating the toxicologic potential of an environmental sample or a complex mixture, the concentration of each congener present multiplied by its equivalency factor gives the concentration of the congener in terms of the TCDD equivalent. The potential effect of the sample or mixture is expressed by the sum of the TCDD equivalent, as if it were concentration of TCDD itself. Safe (*3*) and Safe et al. (*31*) reviewed several classes of halogenated aromatics and suggested that the TEFs established for the PCDDs and PCDFs can be similarly used for the bromo and bromo/chloro dibenzo-*p*-dioxins and dibenzofurans.

Comparative Potencies of PCDDs and PBDDs

Nagao et al. (*28*) compared the abilities of TCDD and TBDD to induce hepatic EROD in rats. The compounds were administered in single SC doses (TBDD, 0.006 – 6.0 nmole/kg; TCDD, 0.003 – 10 nmole/kg). Both compounds induced EROD, with the magnitudes of induction and the time courses of effects being identical for the two compounds. On a microgram per kilogram basis, TCDD appeared to be approximately twice as potent as TBDD, but on a molar basis, the compounds are equipotent for the induction of EROD in female Wistar rats. Under the conditions of this experiment, the results indicate that the TCDD equivalency of TBDD, when expressed on a molar basis, is 1.0. When expressed on a microgram per kilogram basis, it is 0.5.

Correlations between *in Vitro* Enzyme Induction Potency and the Production of Weight Loss and Thymic Atrophy

Safe et al. (*30*) assessed the abilities of a total of 27 PCDD, PCDF, PCB, and PBDD congeners to induce AHH, then determined their potencies to induce body weight loss and thymic atrophy. The authors reported excellent correlations between EC_{50} concentrations for enzyme induction and ED_{50} doses for either body weight loss or thymic atrophy ($r > 0.9$). They did not, however, present individual data for the brominated compounds that were studied. Despite this, the results indicate that direct and consistent relationships exist between the potencies of the chemicals to produce these effects. Such relationships are required for the determination of meaningful toxic equivalency factors.

Correlations between *in Vivo* Enzyme Induction and the Production of Weight Loss and Thymic Atrophy

Mason et al. (*27*) compared the abilities of bromo and bromochloro dibenzo-*p*-dioxins to induce AHH and EROD and to cause body weight loss and thymic atrophy in immature male Wistar rats. Rats were administered (unspecified) doses of the congeners by the IP route 14 days before the assay of hepatic enzyme activity and the determination of thymus (and body) weights. The molar ED_{50} values for body weight loss, thymic atrophy, and AHH and EROD induction were calculated. Computations of correlation coefficients revealed clear relationships between the magnitudes of biochemical changes and the toxicologic effects: AHH induction versus body weight loss, $r \geq 0.97$; AHH induction versus thymic atrophy, $r \geq 0.97$; EROD induction versus body weight loss, $r \geq 0.99$; EROD induction versus thymic atrophy, $r \geq 0.98$. The results of these experiments suggest that determining a congener's potency for inducing either AHH or EROD can predict its potency to elicit either body weight loss or thymic atrophy in rats.

Comparison of Cytosolic Binding Affinities and Hepatic Enzyme Induction

Mason et al. (*27*) studied the correlations between the relative binding affinities of PCDDs and PBDDs for rat hepatic cytosolic receptor and in the abilities of the compounds to induce hepatic AHH and EROD activity in rat hepatoma H-4-II E cells in culture. Calculation of correlation coefficients revealed differences in the relative potencies of the compounds in their abilities to bind to the cytosolic receptor and in their abilities to induce hepatic enzymes. Although explanations for this lack of correlation are not immediately obvious, the results suggest that events or factors occurring subsequent to binding to the cytosolic receptor are important with regard to differences between chemicals and their abilities to elicit an effect such as enzyme induction. The authors did find, however, that the binding affinities of TBDD and TCDD for the cytosolic receptor were similar (*27*), supporting the idea that the toxicologic potencies of the two compounds are roughly equivalent on a molar basis.

Computation of TCDD Equivalent of Pyrolized, Brominated Flame Retardants

Zacharewski et al. (*6*) pyrolyzed a series of polybrominated flame retardants and analyzed the pryolysates for the presence of PBDDs and PBDFs. The pyrolysates were found to contain a complex mixture of PBDD and PBDF congeners. The *in vitro* abilities of the pryolysates to induce EROD and AHH in rat hepatoma H-4-II cells in culture were determined and compared to the induction produced by TCDD. Although concentrations of TCDD and the PBDDs and PBDFs used for the experiments were not specified in the paper, the computed ED_{50} values were presented and were used to determine the TCDD equivalent of the pyrolysates. Because this is the only report on this type of study, it is impossible to determine either the reproducibility or the accuracy of the TCDD equivalents estimates. The data are summarized in Table 1.

Table 1. Summary of results of pyrolysis of brominated flame retardants and computations of TCDD equivalents from the results of *in vitro* experiments.

Pyrolized flame retardant	Total PBDDs and PBDFs, ppm	TCDD equivalent, ppm	
		AHH induction	EROD induction
Fire Master 300 BA	10,935	174	194
Fire Master BP-6	2,070	1,400	480
Bromkal 70-5-DE	610,393	2,140	4,680
Bromkal 70-DE	268,477	8,780	6,740
Bromkal GI	547,700	3,920	5,260

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PBDD, polybrominated dibenzo-*p*-dioxin; PBDF, polybrominated dibenzofuran; AHH, aryl hydrocarbon hydroxylase; EROD, ethoxresorufin-*o*-deethylase.

Table 2. Summary of results of computations of TCDD equivalents based on the results of *in vivo* experiments conducted on pyrolysates of two brominated flame retardants (*6*).

Pyrolyzed flame retardant	Total PBDDs and PBDFs, ppm	Effect	Computed TCDD equivalent, ppm
Fire Master BP-6	2070	AHH induction	540
		EROD induction	520
		Body weight loss	760
		Thymic atrophy	1680
Bromkal 70-DE	268,477	AHH induction	5200
		EROD induction	3860
		Body weight loss	6260
		Thymic atrophy	8960

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PBDD, polybrominated dibenzo-*p*-dioxin; PBDF, polybrominated dibenzofuran; AHH, aryl hydrocarbon hydroxylase; EROD, ethoxresorufin-*o*-deethylase.

There was reasonably good correlation between the TCDD equivalents in the two *in vitro* experiments. In the case of Fire Master BP-6, there was a nearly 3-fold difference in computed TCDD equivalents, but differences in estimates for the other pyrolysates ranged from 1.1 (Fire Master 300 BA) to 2.1 (Bromkal 70-5-DE).

The *in vivo* effects of two of the pyrolysates on enzyme induction, body weight loss, and thymic atrophy were also determined, and TCDD equivalents were computed. The ranges of results depicted in Table 2 show the estimated concentrations (ppm) of TCDD that would have produced the same effects that were produced by the pyrolysates.

Zacharewski et al. (*6*) noted that the estimated value of the TCDD equivalent appeared to be dependent, in part, on the response that was measured. They pointed out that the results of each assay could have been influenced by several factors, including pharmacokinetics and metabolism and the interactive effects of the mixtures of PBDDs. Because of these factors, it might be useful to express *in vivo* estimates of the TCDD equivalent in terms of a range of values.

Toxicologic Effects of Major Concern

Reports of human toxicity associated with exposure to PBDDs and PBDFs were not found. In addition, only limited data are available concerning toxicity in animal models. Therefore, it is impossible to make unequivocal statements concerning the toxicologic potential that might be associated with human exposure to these compounds. The limited animal data on the PBDDs and PBDFs and the observed human toxicity of PCDDs and PCDFs allow a suggestion that human health hazards that could be associated with exposures to the brominated analogs include carcinogenicity, developmental toxicity, skin toxicity, hepatotoxicity, gastrointestinal disorders, and immunosuppression.

Carcinogenicity

The International Agency for Research on Cancer (*37*) has classified 2,3,7,8-TCDD as a possible human carcinogen (group 2B). The Agency cited sufficient evidence of carcinogenicity in animals and inconclusive evidence in humans. Since publication of that position in 1987, evidence of carcinogenic effects of TCDD in humans has been accumulating [see, for example, Fingerhut et al. (*4*)]. Given the degree of similarity between the chlorine and bromine congeners it is likely that TBDD also possesses carcinogenic potential.

Developmental Toxicity

The administration of TBDD and TBDF to pregnant mice, at doses that cause no evidence of maternal toxicity, produces cleft palate and hydronephrosis in the offspring (*34*). These developmental malformations are also caused by TCDD and TCDF. Although the mechanism(s) through which these chemicals produce their effects remains unknown, TCDD has been shown to potentiate the teratogenic effect of retinoic acid. It has been suggested that TCDD modulates embryonic responses to growth and differentiation factors (*54*). Although TBDD has not been studied for an interaction with retinoic acid, a similar potentiation would be expected (L.S. Birnbaum, U.S. EPA, personal communication).

Skin Toxicity

The most common and persistent effect of TCDD exposure in humans is the production of dermal toxicity [chloracne, skin hyperpigmentation, and in many victims conjunctivitis and irritation of mucous membranes (*1–3*)]. Pinkerton et al. (*55*) reported that both TBDD and TBDF were active in the dermal rabbit-ear test for acnegenic activity. However, these compounds were approximately 1000 times less potent than TCDD. It appears, therefore, that the brominated compounds have the potential to produce skin lesions in humans, although possibly at higher doses relative to those required for TCDD.

Liver Toxicity

TCDD has been associated with elevated serum levels of certain enzymes as well as the excretion of porphoryins and hypercholesteremia. These signs are all suggestive of liver impairment. BDDs and BDFs are hepatotoxic in laboratory species. These effects are similar to those produced by polychlorinated compounds and include the induction of enzymes and increased liver mass. These effects are not necessarily indicative of the ability of the compounds to produce hepatotoxicity in humans, but they do suggest that the liver is a potential target organ.

Gastrointestinal Effects

Human exposure to TCDD has been associated with complaints of abdominal pain, vomiting, and diarrhea. Administration of TCDD to a few species, such as the monkey and cow, produces hyperplastic changes in the gastrointestinal tract. Such findings are not common in rodents, so it is not surprising that the studies cited in this review revealed no evidence of gastrointestinal effects associated with administration of the brominated compounds. Possible gastrointestinal effects due to PBDD and PBDF exposure cannot be ruled out.

Immunotoxicity

TCDD and TCDF (as well as other representatives of these groups) have been repeatedly demonstrated to possess immunosuppressant activity in laboratory animals, as reviewed by Dean et al. (*56*) and Exon et al. (*57*). Evidence of similar effects in humans, however, is less convincing. It is suspected that TCDD-induced immunosuppression may be mediated through the cytosolic Ah receptor (*56*). Given the similarities between the

chloro and bromo analogs, it is likely that the brominated and mixed PHDDs and PHDFs are also immunoactive compounds.

Suggestions for Future Research

Based on this review of the available literature on PBDDs and PBDFs, several areas for future research could provide important insights into the environmental significance and toxicological importance of this class of compounds. *a*) Improved, rapid quantitative and qualitative analytical procedures are required to better assess the magnitude of environmental contamination by the compounds. Methods should be developed for assay of fumes, water, soil, and biological samples. *b*) Studies of the behavior of the brominated chemicals in the ecosystem should be conducted. Such studies should be designed to determine the stability of the chemicals in the environment and their ability to accumulate and concentrate in the food chain. Preferential accumulation of specific congeners should be assessed. Human fat tssues should also be assayed for presence of the compounds. *c*) Because of the enormous number of mixed halogenated derivatives possible, toxicity equivalency factors should be validated for the members of this family of compounds. TEFs may also be employed to conduct structure–activity relationship studies. *d*) Selected bromo and mixed compounds should be assessed for genotoxic and immunotoxic potential. *e*) Comparative toxicology studies between bromo, chloro, and mixed dibenzo-*p*-dioxins and dibenzofurans should be conducted to gain insight into the role of the individual halogens in determining biological effects. These studies should include single and repeated dosage regimens and include assessments of the biological dispositions of the chemicals. Studies should also be designed to determine effects on reproduction and fetal development and potential carcinogenicity.

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